Mathematical Modelling of the Gene Regulatory Network governing Natural Transformation in *Streptococcus thermophilus*

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Context

The Gram-positive bacterium *Streptococcus thermophilus* has the ability to capture exogenous DNA from its environment and to stably integrate it into its genome. This phenomenon, which is called competence for natural transformation, is very costly for the bacteria from an energetic point of view. This is why the physiological state of competence is both transient and fine-tuned by a complex regulatory network.

In *Streptococcus thermophilus*, competence for natural transformation is regulated by the ComRS signalling system :

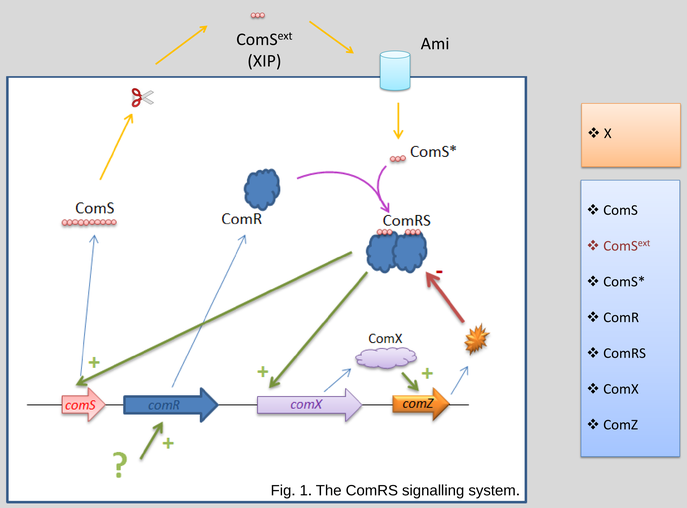

Fig. 1. The ComRS signalling system.

In the current model (1), the peptide pheromone ComS is processed and exported outside the cell (ComSext, a.k.a. XIP), and is next reimported by the oligopeptide transporter Ami. In the cytoplasm, mature ComS (ComS*) directly interacts with the competence regulator ComR. This interaction stimulates the binding activity of ComR dimers on the promoter sequence of *comX,* encoding the alternative sigma factor that controls the expression of genes involved in the uptake and integration of exogenous DNA in the host genome. The ComRS complex also activates the transcription of *comS*, resulting in a **positive feedback loop, which may be essential for the coordination of competence development inside the bacterial population.**

A lot of questions about the ComRS regulatory cascade still remain open. Indeed, it is not known how *comR* is upregulated during competence initiation nor how environmental parameters are integrated to fine-tune the activity of the ComRS complex. Moreover, very little is known about the **shut-off mechanism** of the competence state. In *Streptococcus pneumoniae*, it has recently been shown that the alternative sigma factor activates a protein responsible for both the integration of the ssDNA and the repression of the early phase (2). Similarly, in *Streptoccus thermophilus*, a Δ*comX* strain exhibit a stronger and longer early phase (unpublished data), which could suggest the existence of a similar shut-off mechanism relying on a **negative feedback loop**.

Objective & Strategy

The aim of this study is to develop a dynamic mathematical model of the complex network regulating competence. Based on ordinary differential equations, the model parameters are set according to prior knowledge and experimental data. *In silico* simulations will allow to test different hypothesis and to orient future experiments consequently.

Experimental data is generated by analyzing the specific luciferase activity in different reporter strains. The insertion of an ectopic copy of the promoter of the key early genes (*comR, comS, comX*) upstream of the *luxAB* reporter genes allows us to monitor the specific promoter activities throughout the growth. This time-series is our proxy for the production rates of the corresponding genes. The luciferase activities are also measured in mutant strains, deleted for each early gene.

Another essential information for the model is the growth kinetics, which is estimated by measuring the optical cell density.

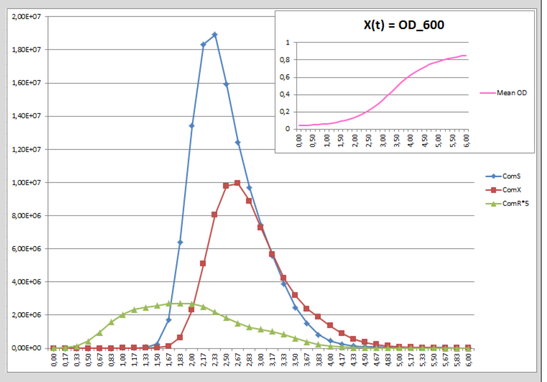

Fig. 2. Growth curve (OD600) and luciferase curves (RLU/OD).

The dynamics of the network regulating competence will be modelled by a set of ordinary differential equations (ODE's). Each equation describes the temporal behaviour of one particular molecular species (Fig. 3), in accordance with the cellular growth equation (Fig. 4).

Since the luciferase curves can be directly used as production rates (green terms), only a few parameters still need to be determined (mainly degradation and transport rates).

While the wild-type luciferase profiles are used to calibrate the parameters (*i.e.* as *learning set*), the profiles from deleted strains will constitute the *validation set*.

Variable	Description	Unit
X(t)	Cell concentration	cell/L
ComS(t)	Number of molecules of precursor ComS (24 a.a.)	mol/cell
$ComS^{ext}$(t)	Concentration of extracellular ComS (7-11 a.a.)	mol/L
$ComS^{*}$(t)	Number of molecules of reimported mature ComS	mol/cell
ComR(t)	Number of molecules of ComR	mol/cell
ComRS(t)	Number of molecules of the complex C_1 : (ComR-$ComS^{*}$)$_2$	mol/cell
ComX(t)	Number of molecules of ComX	mol/cell
ComZ(t)	Number of molecules of the putative repressor of early phase	mol/cell

Fig. 3. Variables description and units.

$$\frac{dX(t)}{dt} = \mu(t)X(t) \tag{1}$$

$$\frac{dComS(t)}{dt} = b_S + \underbrace{a_S^1\left(\frac{ComRS(t)}{\alpha_1 + ComRS(t)}\right)}_{\text{activation by } C_1} - \underbrace{r_{out}ComS(t)}_{\text{exportation}} - \underbrace{d_S ComS(t)}_{\text{degradation}} - \underbrace{\mu(t)ComS(t)}_{growth} \tag{2}$$

$$\frac{dComS^{ext}(t)}{dt} = \underbrace{r_{out}ComS(t)X(t)}_{\text{exportation}} - \underbrace{r_{in}ComS^{ext}}_{\text{importation}} - \underbrace{d_{S^{ext}}ComS^{ext}(t)}_{\text{degradation}} \tag{3}$$

$$\frac{dComS^{*}(t)}{dt} = \underbrace{\frac{r_{in}ComS^{ext}}{X(t)}}_{\text{importation}} - \underbrace{c_1\left(ComR(t)ComS^{*}(t)\right)^n}_{C_1 \text{ formation}} - \underbrace{d_{S^*}ComS^{*}(t)}_{\text{degradation}} - \underbrace{\mu(t)ComS^{*}(t)}_{growth} \tag{4}$$

$$\frac{dComR(t)}{dt} = b_R + \underbrace{a_R^Z\left(\frac{Z(t)}{\alpha_Z + Z(t)}\right)}_{\text{activation by } Z(t)} - \underbrace{c_1\left(ComR(t)ComS^{*}(t)\right)^n}_{C_1 \text{ formation}} - \underbrace{d_R ComR(t)}_{\text{degradation}} - \underbrace{\mu(t)ComR(t)}_{growth} \tag{5}$$

$$\frac{dComRS(t)}{dt} = \underbrace{\frac{c_1}{2}\left(ComR(t)ComS^{*}(t)\right)^n}_{C_1 \text{ formation}} - \underbrace{D(ComZ(t)ComRS)}_{\text{shut-off}} - \underbrace{d_{RS}ComRS(t)}_{\text{degradation}} - \underbrace{\mu(t)ComRS(t)}_{growth} \tag{6}$$

$$\frac{dComX(t)}{dt} = b_X + \underbrace{\alpha_X^1\left(\frac{ComRS(t)}{\alpha_1 + ComRS(t)}\right)}_{\text{activation by } C_1} - \underbrace{d_x ComX(t)}_{\text{degradation}} - \underbrace{\mu(t)ComX(t)}_{growth} \tag{7}$$

$$\frac{dComZ(t)}{dt} = b_Z + \underbrace{a_Z^{S^*}\left(\frac{ComX(t)}{\alpha_Z + ComX(t)}\right)}_{\text{activation by ComX}} - \underbrace{d_Z ComZ(t)}_{\text{degradation}} - \underbrace{\mu(t)ComZ(t)}_{growth} \tag{8}$$

Fig. 4. The system consists in eight equations : blue = growth terms, green = luciferase activities, red = extracellular.

Perspectives

Systems of ordinary differential equations are a powerful tool to model both the structure and the dynamics of a network. Hence it will help us to understand how the different actors involved in competence regulation interact with each other, and to **test different hypothesis** about the regulatory network, leading to new experiments. Moreover, it has been shown that additional regulatory systems directly interfere with the ComRS system. It would therefore be interesting to enlarge our system in order to include the effect of these additional regulators, as well as the effect of environmental parameters such as pH and temperature.

References

1) **Fontaine L. *et al.*** 2013. Mechanism of competence activation by the ComRS signalling system in streptococci. Mol. Microbiol. **87(6)**:1113-32.
2) **Mirouze N. *et al.*** 2013. Direct involvement of DprA, the transformation-dedicated RecA loader, in the shut-off of pneumococcal competence. Proc. Nat. Acad. Sci. U S A. **110(11)**:E1035-44.